REMARKS

Claims 1-24 are currently pending. Claims 2, 5, and 8 are canceled herein. Claims 1, 9, 10, 13, 15, 17, 18, 21, and 24 are amended. The paragraph beginning on page 10, line 17 of the specification has been amended to include sequence identification numbers (SEQ. ID. NOS. 3 and 4) to comply with the sequence rules of 37 CFR 1.821 - 1.825. The sequences found in this paragraph have been amended to correctly reflect the sequence as disclosed in the referenced patents, and thus contain no new matter. A substitute sequence listing and an electronic copy in computer readable form is enclosed in accordance with § 1.825(b).

Support for the amendments to element (a) of claim 1 and to claim 24 can be found on page 12 and in Example 1, pages 17-18, which discuss baculovirus vectors. Support for element (b) of claims 1 and 21 is found on page 13, which discusses the amount of days larvae may be allowed to develop post-infection, prior to harvesting. Support for element (c) of claims 1 and 21 is found on page 13, line 25, and Example 1, page 18, which describe the homogenization of the infected larvae. Support for element (d) of claims 1and 21 is found on pages 13, line 27 to page 14, line 11, which indicate that soluble proteins are in a different fraction of the larval homogenate than membrane proteins, and that a fraction containing the recombinant fusion protein may be isolated by means generally known in the art. Support for element (e) of claims 1 and 21 is found on page 14, lines 25-29, which indicate that the membrane protein may be solubilized. Support for the amendments to element (f) of claims 1 and 21 and to claims 13, 15, and 17 can be found on page 9, which discusses recombinant membrane fusion proteins. Further support for amended claim 21 can be found on page 16, which discusses physically characterizing proteins. Claim 18 has been amended to correct the spelling of "connexin" as suggested by the Office. Claims 9 and 10 have been amended to contain terminology corresponding to the claims from which they depend.

1. Telephone Interview Summary

The Applicant thanks the Examiner for the courtesy of the telephone interview on October 9, 2002. In the interview, the examiner indicated that the Office action that was issued on October 3, 2002 was retracted in favor of a supplemental Office action (the present action, issued October 18, 2002).

2. Rejections under 35 U.S.C. 112, first paragraph

Reconsideration is requested of the rejection of claims 1-4, 6-20, and 24 under 35 U.S.C. §112, first paragraph, for lack of enablement. According to the Office, the specification is enabling with respect to the use of baculovirus vectors for infecting insect larvae, but not with respect to the use of other vectors that infect insect larvae. Although it is maintained that claim 1 is enabled as originally presented, claim 1 has nevertheless been amended in the interests of expediting prosecution to indicate that the larvae are infected with a baculovirus vector. Applicants thus respectfully submit that the basis for rejection of claim 1 and dependent claims 3, 4, 6, 7, 9-20, and 24, on this ground has been removed.

Reconsideration is requested of the rejection of claims 1-4, 6-20, and 24 under 35 U.S.C. §112, first paragraph, for containing subject matter not adequately described in the specification. According to the Office, "the specification has failed to provide a description for the other vectors that can infect insect larvae embraced by the claims" despite the description for a baculovirus vector. As previously noted, claim 1 has been amended in the interests of expediting prosecution to indicate that the larvae are infected with a baculovirus vector. Thus, it is respectfully submitted that the basis for rejection of claim 1 and dependent claims 3, 4, 6, 7, 9-20, and 24, on this ground has been removed.

3. Rejections under 35 U.S.C. §112, second paragraph

Reconsideration is requested of the rejection of claims 1-24 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Office has rejected claim 1 as being confusing as written because it is unclear whether a "recombinant membrane fusion protein or the recombinant membrane protein is intended to be produced and purified." Claim 1 has been amended to indicate that it is a recombinant membrane fusion protein that is produced by the method of claim 1. Applicants thus respectfully submit that the basis for rejection of claim 1 and dependent claims 3-4, 6-7, 9-20, and 24, on this ground has been removed.

Claim 2 has been canceled, thus rendering moot the rejection of claim 2 under §112, 2nd paragraph.

Claim 19 requires the recombinant membrane fusion protein of claim 1 to have biological activity "substantially the same as the native form of the protein." The Office has objected to the term "substantially the same," and has indicated that the definition provided on page 6 of the specification is insufficient "because it is unclear what substantially the same function means." The specification defines "biological activity substantially the same as the native form of the protein" as meaning that "the recombinant fusion protein produced by the method of the current invention is capable of performing substantially the same function as the native form of the protein."[1] Thus, it is clear to one skilled in the art that "substantially the same function" refers to the specific membrane protein function of the native form of the protein. For example, when the recombinant membrane protein is a transport protein, "substantially the same function" refers to membrane transport activity. An example of this is the NCX1 membrane transport protein, described in Example 1. The native form of the NCX1 membrane transport protein is involved in cardiac sodium-calcium exchange, exhibiting bidirectional transport activity depending on the sodium gradient. A recombinant NCX1 membrane transport protein would thus exhibit "biological activity substantially the same as the native form of the protein" when, like the native form of the protein, it exhibits such bidirectional transport activity.[2] Similar examples exist for other types of membrane proteins, such as junctional, cytoskeletal, channel forming, receptor, etc.

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[1] Specification, at page 6, lines 7-9.

[2] *Id.* at pages 20-21, and Figs. 1 and 2.

Claim 20 requires the recombinant membrane fusion protein of claim 1 to have "substantially the same structure as the native form of the protein." The Office has objected to the term "substantially the same," and has indicated that the definition provided on page 6 of the specification is insufficient "because it is unclear what substantially the same tertiary and quaternary structure means."

The specification defines "structurally substantially the same as the native form of the protein" as meaning "the recombinant fusion protein produced by the method of the current invention exhibits substantially the same tertiary and quaternary structure as the native form of the protein."[3] Although not explicitly defined, it would be clear to one skilled in the art that "substantially the same tertiary and quaternary structure" refers to the proper folding of the recombinant membrane protein by the larvae. Proper folding of the recombinant protein is often necessary for the protein to be stable and biologically active, which is one advantage of expressing recombinant proteins in eukaryotic hosts.[4] A recombinant membrane fusion protein would thus have "substantially the same" structure as the native form of the protein if its tertiary and quaternary structure is sufficiently similar to that of the native form of the protein that the recombinant membrane protein is biologically active.

Claim 21 is directed generally to a method for identifying the physical characteristics of a recombinant membrane fusion protein. The Office has rejected claim 21 as being incomplete as written because it "has not provided method steps identify the physical characteristics of a recombinant fusion protein so the goal of the preamble is set forth in a positive process." Applicants respectfully submit that the amendments to claim 21 overcome this objection.

Claim 21 has also been rejected as being indefinite as written "because it is unclear which protein is being produced." Claim 21 has been amended to clarify that the intended protein is a recombinant membrane fusion protein.

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[3] *Id.* at page 6, lines 10-13.

[4] *See, id.* at page 2, lines 13-19.

Given the foregoing, Applicants respectfully request withdrawal of the rejections of claims 1, 3-4, 6-7, and 9-24 under 35 U.S.C. §112, second paragraph.

4. Rejections under 35 U.S.C. 102(b)

Reconsideration is requested of the rejection of claims 21-23 under 35 U.S.C. §102(b) as being anticipated by Pandit, et al. (U.S. 5,866,114).

Claim 21 is directed to a method for identifying the physical characteristics of a recombinant membrane fusion protein. The process comprises (a) infecting insect larvae with a baculovirus vector containing a nucleic acid sequence that encodes a recombinant membrane fusion protein having an affinity tag; (b) allowing the infected larvae to develop for about 1 to 4 days post infection and express the recombinant membrane fusion protein; (c) homogenizing the developed, infected larvae to form a homogenate; (d) separating the homogenate into a membrane-containing portion and a soluble portion, wherein the membrane-containing portion contains the recombinant membrane fusion protein with the affinity tag; (e) solubilizing the separated recombinant membrane fusion protein; (f) purifying the solubilized recombinant membrane fusion protein from said larvae by affinity chromatography; and (g) determining a physical characteristic of the recombinant membrane fusion protein.

In contrast, Pandit describes methods for crystallizing macrophage colony stimulating factor (M-CSF), and also discloses that X-ray crystallography can be used in the characterization of M-CSF. Pandit further discloses that the expression of M-CSF in *E. coli* as opposed to expression in certain eukaryotic hosts is advantageous with regard to crystallization "[b]ecause of the variability of glycosylation and other post-translational modifications present in M-CSF produced in certain eukaryotic hosts."[5] In Example 5, Pandit expresses recombinant human soluble M-CSF receptor in an insect cell expression system.[6] Although Pandit does indicate that some forms of the M-CSF

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[5] Pandit, et al. (U.S. Patent No. 5,866,114), column 6, lines 51-55.

[6] *Id.* at column 13, lines 34-41.

receptor can be recovered in membrane-associated form,[7] only the soluble extracellular domain of the M-CSF receptor was expressed in Example 5. The "tags" in Pandit were thus used to purify the soluble portion of the protein.

In contrast, the protein of claim 21 is a recombinant membrane fusion protein. The specification defines membrane protein as "any protein that is normally an intergral part of or closely associated with a cell membrane."[8] This definition includes those membrane proteins that may comprise, in part, soluble regions. As claim 1 indicates, however, the recombinant membrane fusion protein being produced is located in the membrane-containing portion of the larval homogenate, and *is solubilized* prior to purification.[9] The proteins produced by the method of claim 1 are thus clearly distinct from the soluble extracellular domain of the M-CSF receptor produced in Pandit's Example 5; Pandit's M-CSF receptor does not need to be solubilized, since only the soluble portion is being produced. Given the differences involved in the production of soluble and membrane proteins,[10] this is a distinct difference.

Claims 22 and 23 depend from claim 21 and are not anticipated by Pandit for the same reasons as those identified in connection with claim 21.

5. Rejections under 35 U.S.C. 103(a)

Reconsideration is requested of the rejection of claims 1-13, 19-21, and 24 under 35 U.S.C. §103(a) as being unpatentable over Au-Young et al. (U.S. 5,843,714) taken with Cha, et al. (Biotechnol. Prog., 1999, 15:283-86; hereinafter "Cha et al. (A)"), and Cha, et al. (Biotechnol. Bioeng., 1999, 65:316-24; hereinafter "Cha et al. (B)").

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[7] *Id.* at column 1, lines 64-65.

[8] Specification, page 8, lines 3-4.

[9] *See also* Specification, page 18, Example 1.

[10] *See infra* note 32.

In order to support a prima facie case of obviousness, there must be some suggestion or motivation to modify or combine reference teachings; there must be a reasonable expectation of success; and the references must teach or suggest all of the claim limitations.[11] The rejection must be based upon what the *prior art* would have led one skilled in the art to do. Thus, "[b]oth the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure."[12]

Au-Young et al. describe a human proteolipid (PLHu), and indicate that an insect system can be used to express PLHu.[13] Au-Young et al. also disclose that PLHu can be expressed as a recombinant protein with purification facilitating domains, including metal chelating peptides, protein A domains, and the domain used in the FLAG® affinity purification system.[14] Au-Young et al., however, do not disclose or suggest that such purification facilitating domains can be used to purify ***membrane*** proteins expressed in an insect larvae expression system. More specifically, Au-Young et al. fail to disclose (i) forming a homogenate of the larvae, and separating the homogenate into a membrane-containing portion and a soluble portion, wherein the membrane-containing portion contains the recombinant membrane fusion protein with the affinity tag; (ii) solubilizing the separated recombinant membrane fusion protein; and (iii) purifying the solubilized recombinant membrane fusion protein from said larvae by affinity chromatography, all as required by claim 1.

Although Au-Young et al. indicate that PLHu can be expressed in an insect system, an insect system is merely one of several examples of vector/host systems they

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[11] MPEP §2143; *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

[12] *In re Vaeck*, 20 USPQ2d at 1442.

[13] Au-Young, et al. (U.S. Patent No. 5,843,714), column 9, ln. 56-57.

[14] *Id.* at c. 12, ln. 34-42.

say can be used to express PLHu.[15] Furthermore, of the systems listed, Au-Young et al. discuss fusion proteins with a purification tag in the context of the bacterial systems, not insect systems. For example, with regards to the selection of expression vectors in bacterial systems Au-Young et al. describe several vectors that "direct high level expression of fusion proteins that are readily purified,"[16] such as fusion proteins containing foreign polypeptides expressed with glutathione S-transferase (GST). Au-Young et al. goes on to state "[i]n general, such fusion proteins are soluble and can easily be purified from lysed cells."[17]

In addition, one skilled in the art would be led to the conclusion that the purification facilitating domains (*i.e.*, tags) described by Au-Young et al. are used to purify soluble proteins, rather than membrane-associated proteins. For example, when describing purification facilitating domains, Au-Young et al. state "[o]ther recombinant constructions may join PLHu to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins."[18] In their Example VIII, Au-Young et al. also indicate that PLHu is secreted "into the bacterial growth media which can be used directly in the following assay for activity,"[19] thus implying that the protein produced in Example VIII was not associated with a membrane.[20] This is in contrast to claim 1 of the present invention, which clearly indicates that the protein being purified is a recombinant membrane fusion protein that is located in the membrane-containing portion of the larval

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[15] *Id.* at c. 8-10 (describing bacterial systems, the yeast *Saccharomyces cerevisiae*, plant cell systems, insect system, and animal systems).

[16] *Id.* at c. 9, ln. 13-14.

[17] *Id.* at ln. 25-26 (emphasis added).

[18] *Id.* at c. 12, ln. 29-31 (emphasis added).

[19] *Id.* at c. 27, ln. 11-13.

[20] In Example VIII, Au-Young describes the expression of PLHu in *E. coli*, not a larval system. *Id.* at c. 26, ln. 66-67.

homogenate, and is solubilized prior to purification. This step is not required in the production of soluble proteins, or when producing only a soluble portion of a membrane protein, and is thus not described in Au-Young et al.'s Example VIII.

Au-Young et al.'s Example XI describes the purification of naturally occurring or recombinant PLHu from cells[21] containing PLHu, not larvae.[22] In addition, it is not clear from Example XI whether the PLHu being purified is membrane-associated, or whether it is a soluble portion. Regardless, although Example XI indicates that the whole cell may be solubilized, it does not discuss separately solubilizing a separated recombinant membrane fusion protein.

Cha et al. (A) and (B) disclose the production of soluble recombinant fusion proteins in an insect larval expression system. Like Au-Young et al., Cha et al. (A) and (B) fail to disclose (i) forming a homogenate of the larvae into a membrane-containing portion and a soluble portion, wherein the membrane-containing portion contains the recombinant membrane fusion protein with the affinity tag; (ii) solubilizing the separated recombinant membrane fusion protein; and (iii) purifying the solubilized recombinant membrane fusion protein from said larvae by affinity chromatography, all as required by claim 1. Thus, Cha et al. (A) and (B) do not disclose or suggest the use of insect larvae systems and affinity tags to purify ***membrane*** proteins. Nor do they disclose or suggest that their method could be applied to recombinant membrane fusion proteins, or provide any information from which a person of ordinary skill would conclude that recombinant

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[21] Example XI indicates that cellular fractions of cells containing PLHu can be "prepared by solubilization of the whole cell and isolation of subcellular fractions by differential centrifugation, by the addition of detergent, ro bo other methods well known in the art." *Id.* at c. 28, ln. 19-23.

[22] Cell cultures are fundamentally different from insect larvae. In cell cultures, the cells are all of a uniform type; in contrast, larvae are multicellular, containing many different cell and tissue types. In addition, multicellular larvae contain many more and different proteins than would be found in an individual cell, some of which may interfere with the purification of the recombinant membrane proteins. This concern is not as great when purifying recombinant proteins from cells.

membrane proteins could be successfully expressed and purified in larvae expression systems, simply because they had expressed soluble proteins in insect larvae systems.[23]

In light of the foregoing, applicants submit that claim 1, and dependent claims 3-4, 6-7, 9-13, 19-20, and 24 are patentable over Au-Young et al. taken with Cha et al. A and Cha et al. B, as none of these references suggest the combination of features in claim 1, or provide a reasonable expectation of success.

Claim 21 is directed to a method for identifying the physical characteristics of a recombinant membrane fusion protein comprising (a) infecting insect larvae with a baculovirus vector containing a nucleic acid sequence that encodes a recombinant membrane fusion protein having an affinity tag; (b) allowing the infected larvae to develop for about 1 to 4 days post infection and express the recombinant membrane fusion protein; (c) homogenizing the developed, infected larvae to form a homogenate; (d) separating the homogenate into a membrane-containing portion and a soluble portion, wherein the membrane-containing portion contains the recombinant membrane fusion protein with the affinity tag; (e) solubilizing the separated recombinant membrane fusion protein; (f) purifying the solubilized recombinant membrane fusion protein from said larvae by affinity chromatography; and (g) determining a physical characteristic of the purified recombinant membrane fusion protein. As previously discussed, the cited

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[23] The differences in the production of membrane versus the production of soluble proteins are significant. As previously discussed, soluble proteins are generally free in solution. In contrast, membrane proteins "are a part of or closely associated with a cell membrane and therefore, are typically not free in solution." Specification, p. 1, ln. 23-25. Consequently, the purification of membrane proteins is much more difficult than for soluble proteins. Because of their association with membranes, upon centrifugation membrane proteins are generally located in the pelletable, rather than the soluble, fraction. The association of membrane proteins with the membrane lipid bilayer must be disrupted prior to purification; this is typically done by detergents. It is thus difficult to obtain large quantities of biologically active, purified membrane protein. *Id.* at p. 2, ln. 2-3. Such difficulties are not associated with the purification of soluble proteins, which, because they are generally free in solution, may be readily extracted and purified from the soluble fraction in large quantities that are typically biologically active. *Id.* at p. 1, ln. 20-23.

references do not suggest all these features, in particular (i) forming a homogenate of the larvae into a membrane-containing portion and a soluble portion, wherein the membrane-containing portion contains the recombinant membrane fusion protein with the affinity tag; (ii) solubilizing the separated recombinant membrane fusion protein; and (iii) purifying the solubilized recombinant membrane fusion protein from said larvae by affinity chromatography.

Au-Young et al. and Cha et al. (A) and (B) fail to disclose each of elements (a) to (f) of claim 21 for the reasons stated above with respect to claim 1. They additionally fail to disclose or suggest element (g), *i.e.,* determining a physical characteristic of the purified recombinant membrane fusion protein. According to the Office, Au-Young et al.'s proposed method for assaying the activity of PLHu reads on a method of determining the physical characteristics of PLHu. The assay described by Au-Young et al., however, does not address the physical characteristics of PLHu, but rather relates to monitoring the effect of PLHu on transmembrane pH gradients in liposomes.[24] The Office has also indicated since the GFP marker used by Cha et al. fluoresces under UV light, this is "broadly interpreted to be a method for identifying the physical characteristics of recombinant GFP." Significantly, however, GFP is not a recombinant membrane protein, as is required by claim 21. The cited references have thus not suggested or described every element of claim 21.

In view of the foregoing, it is respectfully submitted that claim 21 is patentable over Au-Young et al, taken with Cha, et al. (A) and (B).

Reconsideration is requested of the rejection of claims 1-12, 15, 19-21, and 24 under 35 U.S.C. §103(a) as being unpatentable over Goli, et al. (U.S. 5,854,411) taken with Cha et al. (A) and Cha et al. (B).

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[24] Au-Young, at c. 27, ln. 15-32.

Goli et al. disclose a human chloride channel (HCCP), and indicate that an insect system can be used to express HCCP.[25] Goli et al. also disclose that sequences encoding HCCP can be joined to purification facilitating domains.[26] Like Au-Young et al., however, Goli et al. do not indicate that such purification facilitating domains can be used to purify ***membrane*** proteins expressed in an insect larvae expression system, and in particular does not disclose (i) forming a homogenate of the larvae, and separating the homogenate into a membrane-containing portion and a soluble portion, wherein the membrane-containing portion contains the recombinant membrane fusion protein with the affinity tag; (ii) solubilizing the separated recombinant membrane fusion protein; and (iii) purifying the solubilized recombinant membrane fusion protein from said larvae by affinity chromatography, all as required by claim 1.

Like Au-Young et al., Goli, et al. merely identify an insect system as one of several examples of vector/host systems that can be used to express a protein.[27] Furthermore, of the systems listed, Goli, et al. discuss fusion proteins with a purification tag in the context of the bacterial systems, not insect systems,[28] and they state that "[i]n general, such fusion proteins are soluble and can easily be purified from lysed cells."[29]

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[25] Goli, et al. (U.S. Patent No. 5,854,411), column 13, lines 16-28.

[26] Goli, et al. indicate such purification facilitating domains include metal chelating peptides, protein A domains, and the domain used in the FLAG® extension/affinity purification system. *Id.* at c. 15, ln. 54-63.

[27] *See id.* at c. 12-13 (describing bacterial systems, the yeast *Saccharomyces cerevisiae*, plant cell systems, insect system, and animal cell systems).

[28] *See id.* at c. 12, ln. 41-43. In addition, Goli states that "[h]ost cells transformed with nucleotide sequences encoding HCCP may be cultured under conditions suitable for the expression and recovery of the protein from cell culture." *Id.* at c. 15, ln. 45-47.

[29] *Id.* at c. 12, ln. 53-54 (emphasis added).

The language used by Goli et al. to describe the purification facilitating domains indicates that such domains are used to purify soluble proteins.[30] Furthermore, Goli et al.'s Example VIII indicates that HCCP is secreted "into the bacterial growth media which can be used directly in the following assay for activity,"[31] thus implying that the protein produced in Example VIII was not associated with a membrane. As previously discussed, the use of purification facilitating domains to purify soluble portions of a protein is in contrast to claim 1 of the present invention, which clearly indicates that the protein being purified is a recombinant membrane fusion protein that is located in the membrane-containing portion of the larval homogenate, and is solubilized prior to purification.

The Office indicates that Goli, et al.'s Example XI relates to a method to purify HCCP involving the use of an immunoaffinity column that comprises a HCCP antibody. Example XI, however, does not describe homogenizing larvae, nor does it describe elements (d) or (e) of claim 1.

For the reasons noted in greater detail above, Cha et al. (A) and (B) also fail to disclose or suggest the use of an insect larvae expression system and affinity tags to purify ***membrane*** proteins,[32] and in particular, they fail to disclose (i) forming a homogenate of the larvae into a membrane-containing portion and a soluble portion, wherein the membrane-containing portion contains the recombinant membrane fusion protein with the affinity tag; (ii) solubilizing the separated recombinant membrane fusion protein; and (iii) purifying the solubilized recombinant membrane fusion protein from said larvae by affinity chromatography, all as required by claim 1.

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[30] "Other recombinant constructions may be used to join sequences encoding HCCP to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins." *Id.* at c. 15, ln. 54-57 (emphasis added).

[31] *Id.* at c. 30, ln. 55-57.

[32] *See supra* note 23.

Claim 21 is directed to a method for identifying the physical characteristics of a recombinant membrane fusion protein. As previously discussed, the cited references do not suggest, in particular, (i) forming a homogenate of the larvae into a membrane-containing portion and a soluble portion, wherein the membrane-containing portion contains the recombinant membrane fusion protein with the affinity tag; (ii) solubilizing the separated recombinant membrane fusion protein; and (iii) purifying the solubilized recombinant membrane fusion protein from the larvae by affinity chromatography. Nor do the cited references describe element (g) of claim 21, determining a physical characteristic of the purified recombinant membrane fusion protein. The Office has indicated that the proposed method for demonstrating the activity of HCCP described in Goli, et al. is interpreted to read on a method of determining the physical characteristics of HCCP. However, Example IX of Goli, et al., does not describe a method for determining the physical characteristic of HCCP, but rather describes a way to confirm the contribution of HCCP to chloride conductance in cells, by using HCCP specific antibodies.[33] The Office has also indicated that since the GFP marker used in Cha fluoresces under UV light, this is "broadly interpreted to be a method for identifying the physical characteristics of recombinant GFP. However, as previously indicated, GFP is not a recombinant membrane protein, as required by claim 21. The cited references have thus not suggested or described every element of claim 21.

In view of the foregoing, it is respectfully submitted that each of the pending claims are patentable over Goli et al. taken with Cha et al. A and B, as none of these references suggest the combination of features in claim 1, or provide a reasonable expectation of success.

Reconsideration is requested of the rejection of claims 1-12, 19-21 and 24 under 35 U.S.C. §103(a) as being unpatentable over Hillman et al. (U.S. 6,033,870) taken with Cha et al. A and Cha et al. B.

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[33] Goli, et al., at c. 31, ln. 15-20.

Hillman et al. describe human integral membrane protein (TMP-2), and indicate that an insect system can be used to express TMP-2.[34] Hillman et al. also disclose that sequences encoding TMP-2 can be joined to purification facilitating domains.[35] Like Au-Young et al. and Goli et al., however, Hillman et al. do not indicate that such purification facilitating domains can be used to purify ***membrane*** proteins expressed in an insect larvae expression system, and in particular does not disclose (i) forming a homogenate of the larvae, and separating the homogenate into a membrane-containing portion and a soluble portion, wherein the membrane-containing portion contains the recombinant membrane fusion protein with the affinity tag; (ii) solubilizing the separated recombinant membrane fusion protein; and (iii) purifying the solubilized recombinant membrane fusion protein from said larvae by affinity chromatography, all as required by claim 1.

Hillman et al., like Au-Young, et al. and Goli, et al. merely identify an insect system as one of several examples of vector/host systems that can be used to express a protein.[36] Furthermore, of the systems listed, Hillman et al. describe fusion proteins with a purification tag in the context of the bacterial systems, not insect systems,[37] and state "[i]n general, such fusion proteins are soluble and can easily be purified from lysed cells."[38]

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[34] Hillman, et al. (U.S. Patent No. 6,033,870), column 15, lines 54-55.

[35] Hillman indicates such purification facilitating domains include metal chelating peptides, protein A domains, and the domain used in the FLAG® extension/affinity purification system. *Id.* at c. 19, ln. 41-50.

[36] *See id.* at c. 15-17 (describing bacterial systems, the yeast *Saccharomyces cerevisiae*, plant cell systems, insect system, and animal cell systems).

[37] *Id.* at c. 16, ln. 12-13. In addition, Hillman, et al. state "[h]ost cells transformed with nucleotide sequences encoding TMP-2 may be cultured under conditions suitable for the expression and recovery of the protein from cell culture." *Id.* at c. 19, ln. 20-23.

[38] *Id.* at c. 16, ln. 24-26 (emphasis added).

The language used by Hillman, et al. to describ the purification facilitating domains also indicates that such domains are used to purify soluble proteins.[39] Furthermore, Hillman et al.'s Example VIII indicates that soluble forms of recombinant TMP-2, including TMP-2 fusion proteins, can be produced by using sequences encoding the extracellular domains of TMP-2.[40] As previously discussed, the use of purification facilitating domains to purify soluble portions of a protein is in contrast to claim 1 of the present invention. In their Example VIII, Hillman et al. also state "[a]lternatively, TMP-2 may be expressed as a membrane-bound protein in a host cell and the recombinant TMP-2 recovered from the membrane of the host cell using techniques well known to the art,"[41] but does not indicate what techniques should be used, or that membrane-bound proteins with an affinity tag should be expressed in an insect larvae system.

The Office indicates that Hillman et al.'s Example XI relates to a method to purify TMP-2 involving the use of an immunoaffinity column that comprises a TMP-2 antibody. Example XI, however, does not describe homogenizing larvae, nor does it disclose elements (d) or (e) of claim 1.

For the reasons noted above, Cha et al. (A) and (B) also fail to disclose or suggeest the use of an insect larvae expression system and affinity tags to purify **membrane** proteins,[42] and in particular, they fail to disclose (i) forming a homogenate of the larvae into a membrane-containing portion and a soluble portion, wherein the membrane-containing portion contains the recombinant membrane fusion protein with the affinity tag; (ii) solubilizing the separated recombinant membrane fusion protein; and

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[39] "Other recombinant constructions may be used to join sequences encoding TMP-2 to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins." *Id.* at c. 19, ln. 41-44 (emphasis added).

[40] *Id.* at c. 34, ln. 36-41.

[41] *Id.* at ln. 47-50 (emphasis added).

[42] *See supra* note 23.

(iii) purifying the solubilized recombinant membrane fusion protein from said larvae by affinity chromatography, all as required by claim 1.

Claim 21 is directed to a method for identifying the physical characteristics of a recombinant membrane fusion protein. As previously discussed, the cited references do not suggest, in particular, (i) forming a homogenate of the larvae into a membrane-containing portion and a soluble portion, wherein the membrane-containing portion contains the recombinant membrane fusion protein with the affinity tag; (ii) solubilizing the separated recombinant membrane fusion protein; and (iii) purifying the solubilized recombinant membrane fusion protein from the larvae by affinity chromatography. Furthermore, the cited references do not describe element (g) of claim 21, determining a physical characteristic of the purified recombinant membrane fusion protein. The Office has indicated that the proposed method for demonstrating the activity of TMP-2 described in Hillman, et al. is interpreted to read on a method of determining the physical characteristics of TMP-2. However, Hillman et al.'s Example IX does not describe a method for determining the physical characteristic of TMP-2, but rather describes a way to demonstrate that increased levels of TMP-2 expression correlates with an increase in metastic potential or increased cell motility.[43] The Office has also indicated that since the GFP marker used in Cha fluoresces under UV light, this is "broadly interpreted to be a method for identifying the physical characteristics of recombinant GFP. However, as previously indicated, GFP is not a recombinant membrane protein, as required by claim 21. The cited references have thus not suggested or described every element of claim 21.

In view of the foregoing, it is respectfully submitted that all pending claims are patentable over Hillman, et al., taken with Cha, et al. A and Cha et al. B.

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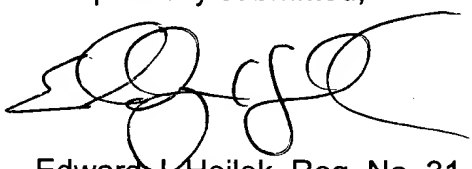
[43] Hillman, et al., at c. 34, ln. 53 to c. 35, ln. 32.

Conclusion

Based on the foregoing, Applicants respectfully request reconsideration of the rejection of claims 1, 3-4, 6-7, and 9-24.

The Commissioner is hereby authorized to charge $465.00 for a three month extension of time to Deposit Account No. 19-1345.

Respectfully submitted,



Edward J. Hejlek, Reg. No. 31,525
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EJH/dep